REMARKS

Claims 1-23, 36, 44, 45, 85-92 and 96-99 constitute the pending claims in the present application. Claims 1, 44, 86, 96 and 99 have been amended. Claims 4, 12-16, 45, 88 and 90-92 have been canceled, without prejudice. Claims 17, 20 and 21 have been withdrawn from consideration. Claims 100-112 have been added. The claim amendments and additions are fully supported by the specification and claims as originally filed. No new matter has been introduced.

Amendment or cancellation of claims should in no way be construed as an acquiescence to any of the Examiner's rejections. The amendments to the claims are being made solely to expedite prosecution of the present application and do not, and are not intended to, narrow the claims in any way. Applicants reserve the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

Claim Rejections Under 35 U.S.C. §103

Claims 44, 45 and 85-89 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Barbas et al. (a) (WO 94/18221) and further in view of Dower et al. (WO 96/40750) and Barbas et al. (b) (PNAS 92: 2529-2533 (1995)) and in view of Kini et al. (FEBS Letters 375: 15-17 (1995)). The rejection is respectfully traversed.

As initial matter, Applicants wish to clarify their position with respect to the Response filed on January 18, 2006. In response to Applicants' arguments regarding the Kini et al. reference and evidence of unexpected results provided in the specification, the Examiner directed Applicants to MPEP §716.01(c)(I) and (II) and concludes that the data presented by Applicant is not found persuasive to be unexpected and unobvious and hence is considered to be moot. The cited portion of the MPEP is directed to probative value of objective evidence and states, at least in part, that "arguments of counsel cannot take the place of evidence in the record" and that "unexpected results must be established by factual evidence." Applicants respectfully request clarification as to the Examiner's position. Applicants arguments at page 12-13 of the Response filed on January 18, 2006 directed the Examiner's attention to factual evidence presented in

Applicant's specification, e.g., evidence on the record. As pointed out in the prior Response, the data provided was a reproduction from the Table in Example 1 of Applicants' specification (see page 45 of the instant application). The Examiner's attention is directed to MPEP §716.01(a) which states that:

> Examiners ***must consider comparative data in the specification*** which is intended to illustrate the claimed invention in reaching a conclusion with regard to the obviousness of the claims. *In re Margolis*, 785 F.2d 1029, 228 USPQ 940 (Fed. Cir. 1986). The lack of objective evidence of nonobviousness does not weigh in favor of obviousness. *Miles Labs. Inc. v. Shandon Inc.*, 997 F.2d 870, 878, 27 USPQ2d 1123, 1129 (Fed. Cir. 1993), *cert. denied*, 127 L. Ed. 232 (1994). However, where a *prima facie* case of obviousness is established, the failure to provide rebuttal evidence is dispositive. (emphasis added)

Applicants respectfully request that the Examiner consider the objective evidence of non-obviousness presented in Applicants' specification (for example, the Table in Example 1 at page 45 of the instant application). The discussion of Applicants' data and how it relates to the teachings of Kini et al. as presented in the Response filed on January 18, 2006 at pages 12-13 is hereby incorporated by reference. The Examiner must consider the data presented in Applicants specification. If such evidence is not found persuasive, a specific explanation as to such reasoning is requested.

The Examiner also stated that the argument of unexpected results was "not commensurate in scope with claims 1-16, 18, 19, 22, 23, 36, 86, 90 and 96-99, because these claims do not require proline at the C-terminus" (see Office Action, page 8, last paragraph). The Examiner also requested clarification as to Applicants' arguments regarding the proline residues in view of claim 86. The Examiner stated that "it is not clear as to the arguments on page 13 (paragraph 2, in particular)...because not all di-peptides as encompassed by claim 86 have prolines in them" (see Office Action, page 7, first full paragraph). Applicants respectfully point out that the prior Response clearly directs the arguments at pages 12-13 to claims 44-45, 85, and 87-89 (see e.g., Response dated January 18, 2006 at page 12, line 5 "With respect to claims 44-45, 85, and 87-89..." to page 13, end of second paragraph "[t]herefore, claims 44-45, 85, and 87-89, are novel and non-obvious in view of the cited references."). Applicants note that claim 86 was addressed in the Response filed January 18, 2006 at page 13, last paragraph to the top of page 15.

Applicants now address the rejection of claims 44, 45 and 85-89 as outlined above (see e.g., pages 4-9 of the Office Action dated March 24, 2006). Applicants note that claims 45 and

88 have been canceled thereby obviating the rejection with respect to those claims. Certain claims will be addressed individually below as noted.

Applicants respectfully disagree with the rejection and note that pursuant to MPEP 2142, "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

With respect to claims 44-45, 85, and 87-89, Applicants respectfully submit that the references cited by the Examiner, taken alone or in any combination, fail to teach or suggest an immunoglobulin molecule, or fragment thereof, wherein one or more amino acid residues of a CDR region are replaced with a biologically active peptide flanked with a proline at the *carboxy terminus*. In particular, the Examiner relies on Kini et al. for the use of prolines to bracket a peptide sequence. However, Kini et al. discloses adding prolines to the ends of *short peptides* and does not teach adding prolines to a peptide that is *inserted into the middle of a large protein* such that the inserted proline has very long flanking sequences, not merely one or two amino acids added to the ends. Additionally, the results obtained by Kini et al. using the short peptides *do not appear to be predictive of the results* obtained when inserting a peptide into the middle of a larger protein. Kini et al. discloses that "[i]ncorporation of a proline residue on either side of RGDM enhances the potency to about the same extent" (see Kini et al. page 16, left column, first full paragraph). In contrast to the teachings of Kini et al., Applicants have found that addition of a proline residue at the carboxy terminus of a peptide which has been incorporated into a CDR region of an antibody provides superior results as compared to incorporation of a proline residue at the amino terminus of the peptide. In particular, the **Table in Example 1** (see page 45 of the instant application) provides the results for various Fab clones having different residues flanking the carboxy terminus and amino terminus of the incorporated peptide. Applicants show that Fab clones having a proline residue immediately flanking the carboxy terminus of an incorporated peptide (e.g., clones X1c, X3a, X3b, X4b, X4c, X5a, X5c, and X7c) resulted in strong binders

whereas Fab clones having a proline residue immediately flanking the amino terminus of an incorporated peptide did not result in strong binders (e.g., clone X1a). Based on the disclosure of Kini et al., one would have expected that the addition of a proline to the amino terminus or carboxy terminus *would have produced equivalent results*. Accordingly, the results obtained by Applicants for incorporation of a peptide into a CDR region are unexpected and nonobvious over the disclosure of Kini et al. relating to short peptides.

Furthermore, neither Barbas (a), Barbas (b) nor Dower make up for the deficiencies of Kini et al. In particular, Barbas (a) discloses immunoglobulins having peptide replaced CDRs and that it may be possible to optimize antibody binding by randomizing residues flanking either side, or both sides, of the incorporated peptide. However, Barbas (a) provides no teaching or suggestion that a *proline* residue flanking the *carboxy terminus* of the peptide would be useful for producing an immunoglobulin having a biologically active peptide incorporated into the CDR region. Additionally, neither Barbas (b) nor Dower suggests addition of a proline residue to the carboxy terminus of a biologically active peptide. Accordingly, no combination of the cited references teaches or suggests the unexpected and superior results obtained from inserting a *proline* residue at the *carboxy terminus* of a peptide that has been incorporated into the CDR region of an immunoglobulin molecule. Therefore, **claims 44-45, 85, and 87-89**, are novel and non-obvious in view of the cited references.

With respect to claim 86, Applicants respectfully submit that the references cited by the Examiner, taken alone or in any combination, fail to teach or suggest an immunoglobulin molecule, or fragment thereof, wherein one or more amino acid residues of a CDR region are replaced with a biologically active peptide flanked at the *carboxy terminus* with the *specifically recited amino acid sequences*. In particular, Barbas et al. (a) teaches CDR replaced antibodies and that optimization of antibody binding may be achieved by *randomizing* residues flanking the incorporated peptide at either terminus. However, Barbas et al. (a) fails to teach or suggest that a dipeptide amino acid sequence should be introduced flanking the peptide, fails to teach or suggest the *specific dipeptide amino acid sequences* provided in claim 86, and fails to teach or suggest that such amino acid sequences should be incorporated at the *carboxy terminus* of the peptide introduced into the CDR region. In particular, Barbas et al. (a) teaches that the flanking regions of the peptide incorporated into the CDR region may be randomized based on the following equation: -X-$[MNN]_a$-Y-$[MNN]_b$-X-, wherein X is a trinucleotide encoding cysteine

or a native amino acid residue coded by the immunoglobulin gene, N is independently any nucleotide, M is adenine (A) or cytosine (C) or analogs thereof, Y is a nucleotide sequence that encodes a minimum recognition domain of the binding site (e.g., the peptide incorporated into the CDR), and *the sum of a and b is from 5 to 50* (see e.g., page 6, lines 10-20). This equation provides an almost endless number of possible combinations of flanking residues for the incorporated polypeptide. In particular, Barbas et al. (a) teaches that the minimum number of flanking residues must be at least 5 amino acids (e.g., a + b = 5). This means that of all of the many possible combinations of flanking sequences provided in Barbas et al. (a), only a few possible permutations provide a dipeptide flanking the carboxy terminus of an incorporated peptide and these would require that the amino terminus has at least three flanking residues (e.g., a is from 3 to 48 and b is 2). Furthermore, of the few possible permutations involving a dipeptide flanking the carboxy terminus there are again many possible dipeptide combinations that would be encompassed by Barbas et al. (a), however, there is no teaching or suggestion of the *particular dipeptide amino acid sequences* listed in claim 86 of the instant application (e.g., proline-valine, proline-aspartic acid, proline-isoleucine, serine-asparagine, serine-lysine, serine-glycine, serine-arginine, leucine-histidine, leucine-glutamic acid, leucine-alanine, leucine-phenylalanine, valine-glutamine, valine-serine, valine-alanine, valine-asparagine, isoleucine-serine, isoleucine-tyrosine, asparagine-proline, asparagine-serine, asparagine-tryptophan, asparagine-valine, phenylalanine-valine, threonine-serine, methionine-alanine, arginine-serine, arginine-glycine, arginine-threonine, arginine-leucine, arginine-valine, tryptophan-arginine, tryptophan-tryptophan, alanine-arginine, aspartic acid-valine, glycine-tyrosine, glutamine-arginine, and glycine-lysine). Accordingly, there is no teaching or suggestion in Barbas et al. (a) for incorporation of a dipeptide amino acid sequence having any of the specific sequences recited in instant **claim 86**. Furthermore, Barbas et al. (b), Dower, and Kini et al. fail to make up for the deficiencies of Barbas et al. (a). In particular, none of the references teaches or suggests that the *specific* dipeptide amino acid sequences provided in **claim 86** should be incorporated at the carboxy terminus of a biologically active peptide that has been introduced into a CDR region. Accordingly, no combination of the cited references teaches or suggests an immunoglobulin molecule, or fragment thereof, wherein one or more amino acid residues of a CDR region are replaced with a biologically active peptide flanked at the *carboxy terminus* with the *specifically*

recited amino acid sequences. Accordingly, **claim 86** is novel and non-obvious in view of the cited references.

Based on the above remarks, Applicants submit that the currently claimed immunoglobulins and fragments thereof are not obvious in view of the cited references. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim Rejections Under 35 U.S.C. §112, second paragraph

Claims 44, 45, and 85-89 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for recitation of the phrase "biologically active peptide." Applicants respectfully traverse the rejection. In particular, the phrase "biologically active peptide" would be clearly understood by one of ordinary skill in the art on its face and/or in light of the teachings of the specification. A biologically active peptide is a peptide that exhibits a biological activity. The specification clearly describes what is meant by a biological activity. For example, at page 13, lines 1-17, the specification defines biological activity. Examples of biological activities are also provided and include, for example, agonist, antagonist, enzymatic, etc. (see e.g., page 4, lines 26-31). Additionally, at page 13, line 8 to page 14, line 28, etc., the specification provides numerous examples of biologically active peptides. Accordingly, one of skill in the art would clearly understand what is meant by the term "biologically active peptide."

Applicants respectfully wish to remind the Examiner that the test for definiteness under 35 U.S.C. §112, second paragraph, is whether "those skilled in the art would understand what is claimed when the claim is read in light of the specification." *See Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565 (Fed. Cir. 1986) and MPEP §2173.02. If the language used by applicant satisfies the statutory requirements of 35 U.S.C. §112, second paragraph, but the examiner merely wants that applicant to improve the clarity or precision of the language used, the claim must not be rejected under 35 U.S.C. §112, second paragraph, rather the examiner should suggest improved language to the applicant (see MPEP §2173.02). Additionally, breadth of a claim is not to be equated with indefiniteness. *See In re Miller*, 441 F.2d 689 (CCPA 1971) and MPEP §2173.04. If the scope of the subject matter embraced by the claims is clear then the claims comply with 35 U.S.C. §112, second paragraph. *Supra*. Additionally, the Examiner is required to provide an analysis as to why the phrase used in the claim is vague and indefinite in the Office Action. See MPEP §2173.02.

Accordingly, Applicants respectfully submit that the claims fully comply with the requirements of 35 U.S.C. §112, second paragraph, as one of ordinary skill in the art could clearly interpret the metes and bounds of the claims. Reconsideration and withdrawal of the rejections under 35 U.S.C. §112, second paragraph, are respectfully requested.

Claim Rejections Under 35 U.S.C. §103

Claims 1-16, 18, 19, 22, 23, 36, 44-45, 85-90, and 96-99 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Barbas et al. (a) (WO 94/18221) and further in view of Dower et al. (WO 96/40750) and Barbas et al. (b) (PNAS 92: 2529-2533 (1995)) and in view of Kini et al. (FEBS Letters 375: 15-17 (1995)) and in view of Cwirla et al. (Science 276: 1696-1699 (1997)) and further in view of Wrighton et al. (Science 273: 458-463 (1996)) as evidenced by Helms (Protein Science 4: 2073-2081 (1995)). Applicants respectfully traverse this rejection.

As an initial matter, Applicants note with appreciation the Examiner's statement at page 3 of the Office Action indicating that the rejection of claims 1-16, 18, 19, 22, 23, 36, 90 and 96-99 under 35 U.S.C. §103(a) over Barbas et al. (a), Dower et al., Barbas et al. (b) and Kini et al. in view of the amendments to the claims to specify that the immunoglobulin molecule or fragment thereof *binds to and agonizes* an EPO or TPO receptor. However, Applicants respectfully request clarification. As outlined above, the Examiner has merely added two additional references to the rejection but it appears to be the same rejection (see pages 10-16 of the Office Action). The new references, Cwirla et al. and Wrighton et al., merely disclose TPO or EPO peptides or mimetics. It has not been explained how these references change or advance the argument in comparison to the previous rejection which was withdrawn. Applicants note that the same arguments which were sufficient to overcome the prior rejection of claims 1-16, 18, 19, 22, 23, 36, 90 and 96-99 apply equally to the current rejection and therefore the rejection should be withdrawn for the same reasons.

Applicants now address the rejection of claims 1-16, 18, 19, 22, 23, 36, 44-45, 85-90, and 96-99 as outlined above (see e.g., pages 10-16 of the Office Action dated March 24, 2006). Applicants note that claims 4, 12-16, 45, 88 and 90-92 have been canceled thereby obviating the rejection with respect to those claims. Certain claims will be addressed individually below as noted.

The Examiner relies on Barbas (a) for allegedly disclosing replacing CDRs in a heavy or light chain of an antibody of Fab fragment with biologically active peptides and randomizing the flanking sequences for presenting a biologically active peptide in a conformation for binding to a receptor. Dower is relied on for disclosure of TPO mimetics. Barbas (b) is relied on for allegedly disclosing replacement of CDR3 in the anti-tetanus toxoid antibody with several sequences. Kini is relied on for disclosing the design of biologically active peptides with proline residues flanking the sequence. Cwirla is relied on for disclosing a TPO peptide that can act as an agonist. Wrighton is relied on for teaching small peptides that can act as EPO mimetics. Helms is relied on for disclosing that proline residues decrease the conformational flexibility of a peptide and thus would constrain the peptide.

Applicants respectfully disagree with the rejection and note that pursuant to MPEP 2142, "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

With respect to claims 1-16, 18-19, 22-23, 36, 90, and 96-99, Applicants respectfully submit that the references cited by the Examiner, taken alone or in any combination, fail to teach or suggest an immunoglobulin molecule, or fragment thereof, wherein one or more amino acid residues of a CDR region are replaced with an agonist peptide (such as an EPO or TPO mimetic) that binds to and agonizes a receptor (such as an EPO or TPO receptor) as claimed in the instant application. In particular, Barbas (a) states that the antibodies described in the application "are particularly well suited for *in vivo* use as a therapeutic reagent for blocking or inhibiting the function of the target molecule which the antibody binds" (see e.g., page 75, lines 29-34). Barbas (a) further provides specific examples of methods for inhibiting platelet gpIIb/IIIa function, methods for inhibiting HIV gp120-mediated events, and methods for inhibiting vitronectin receptor-mediated events (see e.g., pages 78-83) using the CDR replaced antibodies. Accordingly, Barbas (a) teaches methods for designing and using CDR replaced antibody

molecules that *inhibit* or *antagonize* receptor function, e.g., by binding to the receptor and interfering with ligand binding. There is no teaching or suggestion in Barbas (a) that CDR replaced antibodies could be used to *stimulate* or *agonize* receptor function and/or receptor mediated events. One of ordinary skill in the art would clearly understand that Barbas (a) only discusses antagonists in contrast to the pending claims which are directed to agonists. Applicants have attached hereto as Exhibit A an excerpt from the textbook *Molecular Cell Biology*, H. Lodish et al. eds., W.H. Freeman & Co., New York, NY (3d ed., 1995) which describes (on page 871, right hand column) the difference between an agonist and an antagonist:

> Studies with chemically synthesized analogs of epinephrine and other natural hormones have provided additional evidence that saturable cell-surface receptors are physiologically relevant. These analogs fall into two classes: *agonists*, which mimic the function of a hormone by binding to its receptor and causing the normal response, and *antagonists*, which bind to the receptor but do not activate hormone-induced effects. An antagonist acts as an inhibitor of the natural hormone (or agonist) by competing for binding sites on the receptor, thereby blocking the physiological activity of the hormone.

A clear distinction is drawn between an agonist, which activates, and an antagonist, which blocks.

The additional references cited by the Examiner fail to make up for the deficiencies of Barbas (a). In particular, Barbas (b) discloses anti-tetanus toxoid Fab molecules that are CDR replaced and bind to DNA. Such antibodies do not even bind to a receptor let alone suggest that such antibodies could be used to *agonize* receptor activity. Furthermore, there would be no motivation for one of skill in the art to combine the teachings of Barbas (a) with the teachings of Dower, Cwirla, or Wrighton. Specifically, Barbas (a) teaches methods for designing and using CDR replaced antibodies that *antagonize* receptor function while Dower and Cwirla disclose TPO *agonist* peptides and Wrighton discloses EPO mimetics. One of skill in the art would not be motivated to incorporate peptides whose therapeutic value comes from the ability to stimulate receptor activity into a system useful for designing antibodies that inhibit receptor function. Furthermore, Applicants note that both Cwirla and Wrighton are directed to the discovery of *small peptides* that can be used as agonists of the EPO or TPO receptor. In particular, both reference utilize a phage display library to isolate peptides that *bind* to the desired receptor. These peptides are then synthesized as *isolated peptides* and tested for receptor agonist activity. The peptides are not tested for receptor agonist activity in the context of the phage display. In

particular, Wrighton notes that "[t]his discovery may form the basis for the design of *small molecule* mimetics of EPO" (see abstract; emphasis added) and that "*small molecule* EPO mimetics may have desirable pharmacological properties such as oral bioavailability or the ability to be delivered trans-dermally" (see page 463, emphasis added). Why would one of skill in the art want to incorporate the peptides of, for example, Wrighton, into a much larger antibody molecule when Wrighton teaches that the goal is develop small molecule therapeutics for their desirable pharmacological properties? This would be doing exactly the opposite of the teachings of Wrighton. Therefore, one of ordinary skill in the art would not be motivated to incorporate the *small, agonist peptides*, such as the TPO peptides of Dower, Cwirla and Wrighton into the CDR replaced antibodies of Barbas (a) or (b). Furthermore, none of the references provide a suggestion that such a combination should be made nor that such a combination would have any therapeutic utility.

Finally, Kini et al. and Helms et al. fail to make up for the deficiencies of Barbas (a) and (b), Dower, Wrighton and Cwirla. Kini merely proposes that proline residues flanking protein-protein interaction sites perform a structural role in enhancing their interaction. The Helms reference is directed to stability and conformational effects of the introduction of sequences into CDR regions. However, both Kini and Helms fail to teach or suggest that an agonist peptide (such as EPO or TPO) should be incorporated into the CDR region of an immunoglobulin or a fragment thereof.

Accordingly, none of the references cited by the Examiner, taken alone or in any combination teach or suggest the CDR replaced antibodies as claimed in the instant application. No combination of the cited references teaches inserting agonist peptides into an immunoglobulin wherein the resulting immunoglobulin has an agonistic activity. Rather, the cited references merely teach immunoglobulins which bind and block receptors and therefore are antagonists. In particular, the references relied on by the Examiner merely teach CDR replaced inhibitory antibodies and TPO peptides that are useful as agonists. However, one of ordinary skill in the art would have no motivation to incorporate such agonist peptides into the CDR replaced antibodies disclosed to be useful as receptor inhibitory agents. Therefore, **claims 1-16, 18-19, 22-23, 36, 90, and 96-99** are novel and non-obvious in view of the cited references.

With respect to claims 44-45, 85, and 87-89, Applicants respectfully submit that the references cited by the Examiner, taken alone or in any combination, fail to teach or suggest an

immunoglobulin molecule, or fragment thereof, wherein one or more amino acid residues of a CDR region are replaced with a biologically active peptide flanked with a proline at the *carboxy terminus*. In particular, the Examiner relies on Kini et al. for the use of prolines to bracket a peptide sequence. However, Kini et al. discloses adding prolines to the ends of *short peptides* and does not teach adding prolines to a peptide that is *inserted into the middle of a large protein* such that the inserted proline has very long flanking sequences, not merely one or two amino acids added to the ends. Additionally, the results obtained by Kini et al. using the short peptides *do not appear to be predictive* of the results obtained when inserting a peptide into the middle of a larger protein. Kini et al. discloses that "[i]ncorporation of a proline residue on either side of RGDM enhances the potency to about the same extent" (see Kini et al. page 16, left column, first full paragraph). In contrast to the teachings of Kini et al., Applicants have found that addition of a proline residue at the carboxy terminus of a peptide which has been incorporated into a CDR region of an antibody provides superior results as compared to incorporation of a proline residue at the amino terminus of the peptide. In particular, the **Table in Example 1** (see page 45 of the instant application) provides the results for various Fab clones having different residues flanking the carboxy terminus and amino terminus of the incorporated peptide. Applicants have shown that Fab clones having a proline residue immediately flanking the carboxy terminus of an incorporated peptide (e.g., clones X1c, X3a, X3b, X4b, X4c, X5a, X5c, and X7c) resulted in strong binders. This includes clones with proline only at the carboxy terminus and not at the amino terminus (X1c, X3a, X4c, X5a, X5c and X7c), whereas Fab clones having a proline residue immediately flanking the amino terminus but not at the carboxy terminus of an incorporated peptide did not result in strong binders (e.g., clone X1a). Based on the disclosure of Kini et al., one would have expected that the addition of a proline to the amino terminus or carboxy terminus *would have produced equivalent results*. Accordingly, the results obtained by Applicants for incorporation of a peptide into a CDR region are unexpected and nonobvious over the disclosure of Kini et al. relating to short peptides.

Furthermore, none of Barbas (a), Barbas (b), Dower, Wrighton, Cwirla or Helms make up for the deficiencies of Kini et al. In particular, Barbas (a) discloses immunoglobulins having peptide replaced CDRs and that it may be possible to optimize antibody binding by randomizing residues flanking either side, or both sides, of the incorporated peptide. However, Barbas (a) provides no teaching or suggestion that a *proline* residue flanking the *carboxy terminus* of the

peptide would be useful for producing an immunoglobulin having a biologically active peptide incorporated into the CDR region. Additionally, none of Barbas (b), Dower, Wrighton, Cwirla or Helms suggests addition of a proline residue to the carboxy terminus of a biologically active peptide. Accordingly, no combination of the cited references teaches or suggests the unexpected and superior results obtained from inserting a *proline* residue at the *carboxy terminus* of a peptide that has been incorporated into the CDR region of an immunoglobulin molecule. **Therefore, claims 44-45, 85, and 87-89**, are novel and non-obvious in view of the cited references.

With respect to claim 86, Applicants respectfully submit that the references cited by the Examiner, taken alone or in any combination, fail to teach or suggest an immunoglobulin molecule, or fragment thereof, wherein one or more amino acid residues of a CDR region are replaced with a biologically active peptide flanked at the *carboxy terminus* with the *specifically recited amino acid sequences*. In particular, Barbas et al. (a) teaches CDR replaced antibodies and that optimization of antibody binding may be achieved by randomizing residues flanking the incorporated peptide at either terminus. However, Barbas et al. (a) fails to teach or suggest that a dipeptide amino acid sequence should be introduced flanking the peptide, fails to teach or suggest the *specific dipeptide amino acid sequences* provided in claim 86, and fails to teach or suggest that such amino acid sequences should be incorporated at the *carboxy terminus* of the peptide introduced into the CDR region. In particular, Barbas et al. (a) teaches that the flanking regions of the peptide incorporated into the CDR region may be randomized based on the following equation: -X-$[MNN]_a$-Y-$[MNN]_b$-X-, wherein X is a trinucleotide encoding cysteine or a native amino acid residue coded by the immunoglobulin gene, N is independently any nucleotide, M is adenine (A) or cytosine (C) or analogs thereof, Y is a nucleotide sequence that encodes a minimum recognition domain of the binding site (e.g., the peptide incorporated into the CDR), and *the sum of a and b is from 5 to 50* (see e.g., page 6, lines 10-20). This equation provides an almost endless number of possible combinations of flanking residues for the incorporated polypeptide. In particular, Barbas et al. (a) teaches that the minimum number of flanking residues must be at least 5 amino acids (e.g., a + b = 5). This means that of all of the many possible combinations of flanking sequences provided in Barbas et al. (a), only a few possible permutations provide a dipeptide flanking the carboxy terminus of an incorporated peptide and these would require that the amino terminus has at least three flanking residues (e.g.,

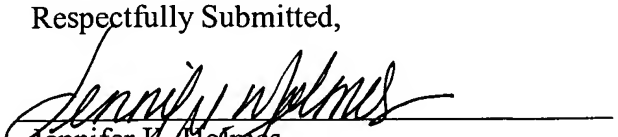
a is from 3 to 48 and b is 2). Furthermore, of the few possible permutations involving a dipeptide flanking the carboxy terminus there are again many possible dipeptide combinations that would be encompassed by Barbas et al. (a), however, there is no teaching or suggestion of the particular dipeptide amino acid sequences listed in claim 86 of the instant application (e.g., proline-valine, proline-aspartic acid, proline-isoleucine, serine-asparagine, serine-lysine, serine-glycine, serine-arginine, leucine-histidine, leucine-glutamic acid, leucine-alanine, leucine-phenylalanine, valine-glutamine, valine-serine, valine-alanine, valine-asparagine, isoleucine-serine, isoleucine-tyrosine, asparagine-proline, asparagine-serine, asparagine-tryptophan, asparagine-valine, phenylalanine-valine, threonine-serine, methionine-alanine, arginine-serine, arginine-glycine, arginine-threonine, arginine-leucine, arginine-valine, tryptophan-arginine, tryptophan-tryptophan, alanine-arginine, aspartic acid-valine, glycine-tyrosine, glutamine-arginine, and glycine-lysine). Accordingly, there is no teaching or suggestion in Barbas et al. (a) for incorporation of a dipeptide amino acid sequence having any of the specific sequences recited in instant claim 86. Furthermore, Barbas et al. (b), Dower, Wrighton, Cwirla, Kini and Helms fail to make up for the deficiencies of Barbas et al. (a). In particular, none of the references teaches or suggests that the specific dipeptide amino acid sequences provided in claim 86 should be incorporated at the carboxy terminus of a biologically active peptide that has been introduced into a CDR region. Accordingly, no combination of the cited references teaches or suggests an immunoglobulin molecule, or fragment thereof, wherein one or more amino acid residues of a CDR region are replaced with a biologically active peptide flanked at the *carboxy terminus* with the *specifically recited amino acid sequences*. Accordingly, **claim 86** is novel and non-obvious in view of the cited references.

Based on the above remarks, Applicants submit that the currently claimed immunoglobulins and fragments thereof are not obvious in view of the cited references. Reconsideration and withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should any additional extensions of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945.**

Respectfully Submitted,



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MOLECULAR CELL BIOLOGY

THIRD EDITION

Harvey Lodish
David Baltimore
Arnold Berk
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Paul Matsudaira
James Darnell

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TABLE 20-5 Metabolic Responses to Hormone-Induced Rise in cAMP in Various Tissues

Tissue	Hormone Inducing Rise in cAMP	Metabolic Response
Adipose	Epinephrine; ACTH; glucagon	Increase in hydrolysis of triglyceride; decrease in amino acid uptake
Liver	Epinephrine; norepinephrine; glucagon	Increase in conversion of glycogen to glucose; inhibition of synthesis of glycogen; increase in amino acid uptake; increase in gluconeogenesis (synthesis of glucose from amino acids)
Ovarian follicle	FSH; LH	Increase in synthesis of estrogen, progesterone
Adrenal cortex	ACTH	Increase in synthesis of aldosterone, cortisol
Cardiac muscle cells	Epinephrine	Increase in contraction rate
Thyroid	TSH	Secretion of thyroxine
Bone cells	Parathyroid hormone	Increase in resorption of calcium from bone
Skeletal muscle	Epinephrine	Conversion of glycogen to glucose
Intestine	Epinephrine	Fluid secretion
Kidney	Vasopressin	Resorption of water
Blood platelets	Prostaglandin I	Inhibition of aggregation and secretion

Source: E. W. Sutherland, 1972, *Science* 177:401.

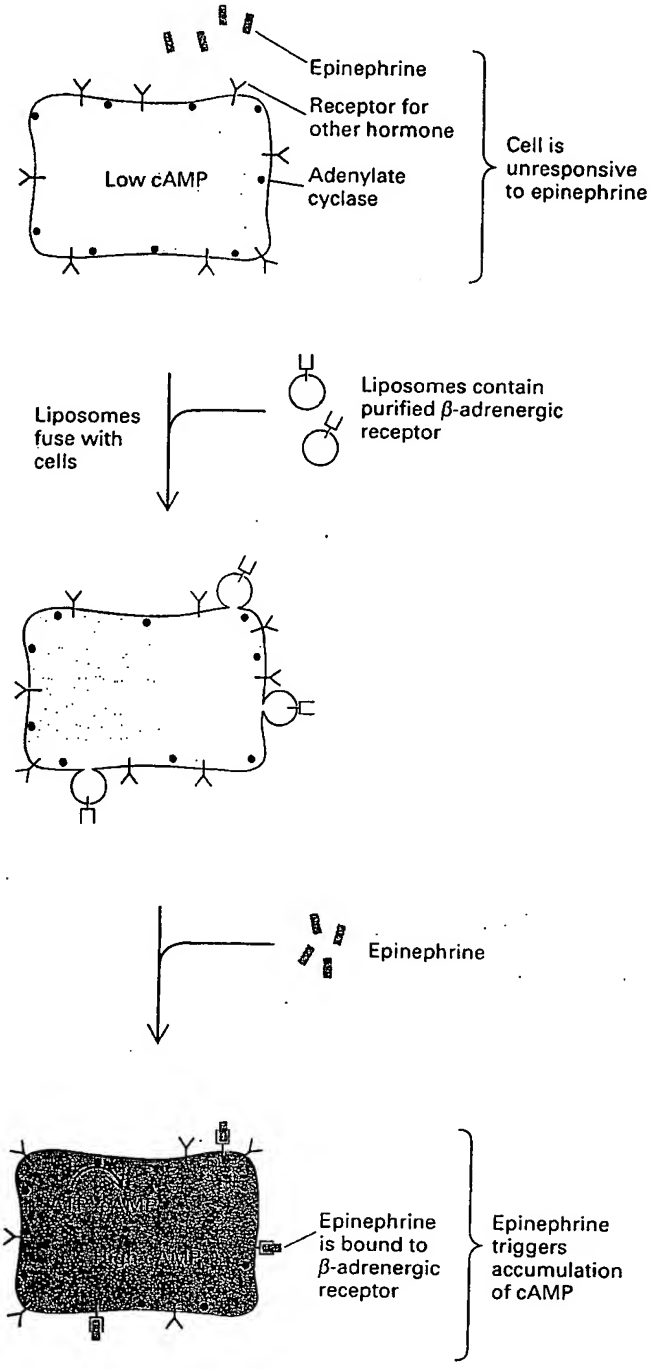
purified receptors were incorporated into liposomes, which then were fused with cells containing adenylate cyclase and the appropriate G proteins but no β-adrenergic receptors (Figure 20-12). Cells that incorporated receptor-laden liposomes responded to epinephrine by synthesizing high levels of cAMP, proving that the receptor is involved in inducing cAMP synthesis. Similar results have been obtained by transfecting cloned cDNA encoding the β-adrenergic receptor into receptor-negative cells; the transfected cells acquire the ability to activate adenylate cyclase in response to epinephrine.

◀ FIGURE 20-11 Comparison of the abilities of three catecholamines to activate adenylate cyclase, which catalyzes synthesis of cAMP, and to bind to cell-surface β-adrenergic receptors. (a) Different concentrations of norepinephrine, epinephrine, and isoproterenol (an agonist) were incubated with a suspension of frog erythrocytes at 37°C. The cells then were broken and the adenylate cyclase activity determined. (b) Ligand binding to the receptor was measured by an indirect competition assay. In this procedure, binding of the unlabeled hormone or agonist to the receptor inhibits binding of the ^{3}H-labeled antagonist alprenolol, allowing the binding affinities of the unlabeled ligands for the receptor to be estimated. The curves show that each ligand induces adenylate cyclase activity in proportion to its ability to bind to the receptor. Moreover, the concentration required for half-maximal binding of each ligand to the receptor is about the same as that required for activation of adenylate cyclase. Note that the ligand concentration is plotted on a logarithmic scale ranging from 10^{-9} to 10^{-2} M.

Analogs Provide Information about Essential Features of Hormone Structure and Are Useful as Drugs

Studies with chemically synthesized analogs of epinephrine and other natural hormones have provided additional evidence that saturable cell-surface receptors are physiologically relevant. These analogs fall into two classes: *agonists*, which mimic the function of a hormone by binding to its receptor and causing the normal response, and *antagonists*, which bind to the receptor but do not activate hormone-induced effects. An antagonist acts as an inhibitor of the natural hormone (or agonist) by competing for binding sites on the receptor, thereby blocking the physiological activity of the hormone.

Comparisons of the molecular structure and activity of various catecholamine agonists and antagonists (Table 20-6) have been used to define the parts of the hormone molecule necessary for binding to β-adrenergic receptors as well as the parts necessary for the subsequent induction of a cellular response. Such studies indicate that the side chain containing the NH group determines the affinity of the



▲ FIGURE 20-12 Experimental demonstration that β-adrenergic receptor mediates the induction of epinephrine-initiated cAMP synthesis. Target cells lacking any receptors for epinephrine but expressing adenylate cyclase and the appropriate signal-transducing G proteins were incubated with liposomes containing purified β-adrenergic receptors. Cells that fused with the liposomes became responsive to epinephrine, producing high levels of cAMP when the hormone was added to the medium. [See R. A. Cerione et al., 1983, *Nature* **306**:562.]

ligand for the receptor, while the catechol ring is required for the ligand-induced increase in cAMP level.

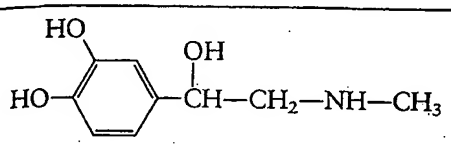
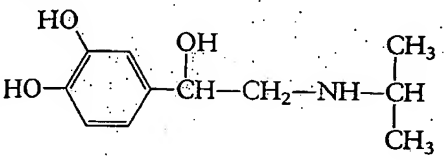
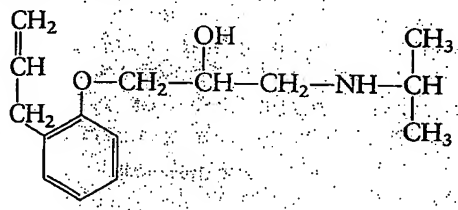
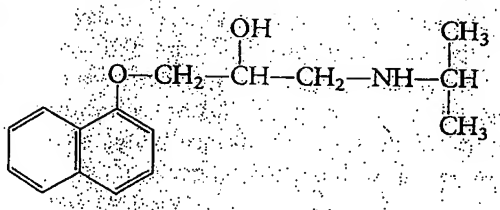
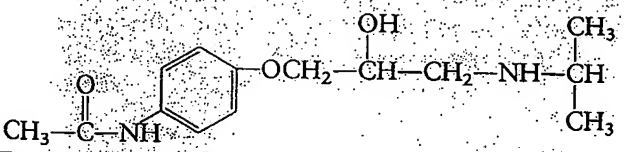
As is true for epinephrine, the K_D for binding of an agonist to β-adrenergic receptors generally is the same as the concentration required for half-maximal elevation of cAMP (see Figure 20-11). This relationship indicates that activation of adenylate cyclase by the epinephrine agonist *isoproterenol* is proportional to the number of β-adrenergic receptors filled with the agonist. Interestingly, the K_D for binding of isoproterenol to β-adrenergic receptors and subsequent induction of cAMP synthesis is 10 times lower than the K_D for epinephrine; other agonists are even more potent with still lower K_D values. The affinity of various antagonists for the β-adrenergic receptor also varies over a wide range (see Table 20-6).

Two types of β-adrenergic receptors have been identified in humans. Cardiac muscle cells possess β_1 *receptors,* which promote increased heart rate and contractility by binding catecholamines with the rank order of affinities isoproterenol > norepinephrine > epinephrine. So-called beta blockers, such as practolol (see Table 20-6), are used to slow heart contractions in the treatment of cardiac arrhythmias and anginas. These β_1-selective antagonists usually have little effect on β-adrenergic receptors on other cell types. The smooth muscle cells lining the bronchial passages possess β_2 *receptors,* which mediate relaxation by binding catecholamines with the rank order of affinities isoproterenol ≫ epinephrine > norepinephrine. Agonists selective for β_2 receptors, such as terbutaline, are used in the treatment of asthma because they specifically mediate opening of the bronchioles, the small airways in the lungs.

Studies with Mutant β-Adrenergic Receptors Identify Residues That Interact with Catecholamines

Mutant forms of the β-adrenergic receptor generated by site-specific mutagenesis have been expressed in cultured cells and their ability to bind the agonist isoproterenol determined. Based on such studies, the model shown in Figure 20-13 has been proposed. Isoproterenol is nestled among the seven transmembrane α helices near the outer (extracellular surface) of the membrane and roughly parallel to the plane of the membrane. Two serine residues in helix *5* of the receptor are hydrogen bonded to the two hydroxyl groups on the catechol ring. Mutation of either of these serines to alanine greatly reduces the ability of the receptor to bind the agonist. The carboxylate group of an aspartate in helix 3 forms an ionic bond with the $-NH_3^+$ group on the catecholamine. Mutation of this aspartate to alanine also inhibits ligand binding by the receptor. Finally, a phenylalanine in helix 6 forms hydrophobic interactions with the catechol ring of the agonist.

TABLE 20-6 Structure of Typical Agonists and Antagonists of the β-Adrenergic Receptor

Structure	Compound	K_D for Binding to the Receptor on Frog Erythrocytes
	Epinephrine	5×10^{-6} M
AGONIST 	Isoproterenol	0.4×10^{-6} M
ANTAGONISTS 	Alprenolol	0.0034×10^{-6} M
	Propranolol	0.0046×10^{-6} M
	Practolol	21×10^{-6} M

Source: R. J. Lefkowitz et al., 1976, *Biochim. Biophys. Acta* 457:1.

Experiments described in a later section provide evidence that the loop connecting helices 5 and 6 bind to the signal-transducing G proteins. Binding of ligand to a β-adrenergic receptor is thought to cause several of the transmembrane helices, particularly helices 5 and 6, to move relative to each other. As a result, the conformation of the long cytosolic loop connecting these two helices changes in a way that allows this loop to bind and activate the transducing G proteins.

Trimeric Signal-Transducing G_s Protein Links β-Adrenergic Receptors and Adenylate Cyclase

As explained already, β-adrenergic receptors on different types of mammalian cells mediate distinct tissue-specific responses (see Table 20-5), but the initial response following binding of epinephrine is always the same: an elevation in the intracellular level of cAMP (Figure 20-14). This in-